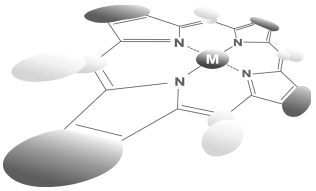
Journal of Porphyrins and Phthalocyanines
J. Porphyrins Phthalocyanines 2023; **27**: 627–633
DOI: 10.1142/S1088424623500463

Published at https://www.worldscinet.com/jpp/



Hydrogenobinamide and nibinamide - Metal-free ligand and Ni(II)-analogue of the vitamin B_{12} precursor cobinamide

Christoph Kieninger[a], Evelyne Deery[b], Andrew D. Lawrence[b], Martin J. Warren[b,c,◊] and Bernhard Kräutler[a,◊,*]

[a]*Institute of Organic Chemistry and Center for Molecular Biosciences (CMBI), University of Innsbruck, 6020 Innsbruck, Austria*
[b]*School of Biosciences, University of Kent, Canterbury, CT2 7NJ. U.K*
[c]*Food Innovation and Health Programme, Quadram Institute of Bioscience, Norwich Research Park, NR4 7UQ. U.K*

Received 10 December 2022
Accepted 31 January 2023

Dedicated to Prof. Tomás Torres on the occasion of his 70th birthday

ABSTRACT: The replacement of cobalt in vitamin B_{12} derivatives by other transition metals is a formal path to non-natural corrins. Here, we describe nibinamide (**Nibi**), the novel Ni(II)-analogue of the natural B_{12}-derivative cobinamide (**Cbi**), and its synthesis from the metal-free ligand of **Cbi**, hydrogenobinamide (**Hbi**), both isolated as tetrafluoroborate salts. Aqueous solutions of the metal-free corrin **Hbi** are strongly fluorescent, whereas its Ni(II)-complex **Nibi** is non-luminescent. The solution structures of **Hbi** and of **Nibi** were characterized by hetero-nuclear NMR-spectroscopy. The Ni(II)-corrin **Nibi** was deduced to be roughly *iso*-structural to cob(I)inamide (**Cbi^{I}**) and to house a diamagnetic d^8-metal-ion *iso*-electronic to Co^{I} in **Cbi^{I}**. The chemically robust **Nibi** is, thus, a structural mimic of enzyme-activated and reduced biosynthetic precursors of vitamin B_{12} and a B_{12}-antimetabolite potentially functioning as a specific inhibitor of B_{12}-biosynthesis.

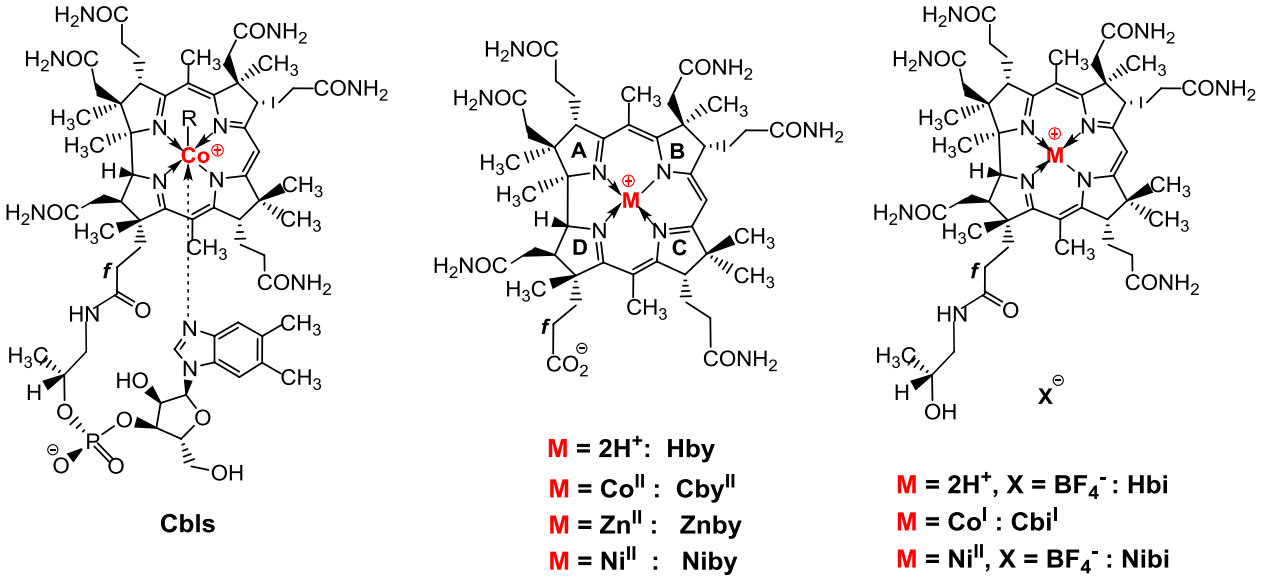
KEYWORDS: antivitamin B_{12}, B_{12}-antimetabolite, corrin, tetrapyrrole, transition metal, vitamin B_{12}.

INTRODUCTION

The pre-eminent biological use of the corrin ligand in the natural vitamin B_{12}-derivatives, and of cobalt as their specific transition metal center, poses the intriguing problem as to why this particular partnership has evolved for providing the unique biochemical reactivity of the B_{12}-cofactors [1–5]. The specific chemistry of cobalt and other transition metals, when bound by the helical 'ring-contracted' natural corrin ligand, is the subject of fundamental questions [1, 2, 6, 7]. Indeed, the synthesis of transition metal analogues of the natural cobalt-corrinoids has been a longstanding 'holy grail' in the B_{12}-field [8–11]. Fortunately, the elucidation of the biochemical B_{12}-biosynthesis paths [12, 13], coupled with bioengineering approaches [14], has opened up direct preparative access to hydrogenobyric acid (**Hby**) [7], the metal-free corrin ligand of vitamin B_{12} (**CNCbl**). The biosynthetic availability of **Hby** has generated a consummate opportunity for the synthesis and characterization of transition metal corrins, including zincobyric acid (**Znby**) [15] and nibyric acid (**Niby**) [16], the Zn(II)- and Ni(II)-complexes of **Hby**, respectively. Furthermore, **Znby** and **Hby** served as rational precursors for the preparation of the 'complete'

◊SPP full member in good standing.

*Correspondence to: Bernhard Kräutler, e-mail. bernhard.kraeutler@uibk.ac.at, Institute of Organic Chemistry and Center of Molecular Biosciences, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria, Tel: +43-664-8125275, Fax: +43-512-507-57799



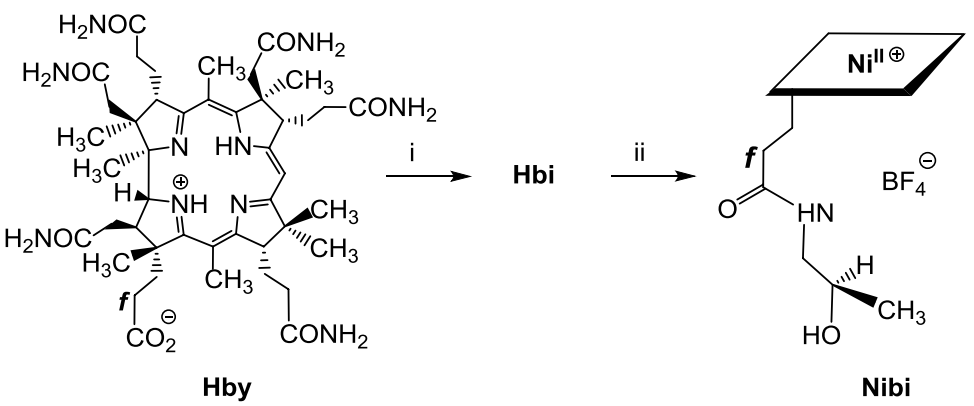
Scheme 1. Formulae of cobalt, zinc, nickel and metal-free corrinoids. Left: General formula of the cobalamins (Cbls) vitamin B12 (R = CN, CNCbl), coenzyme B12 (R = 5′-deoxyadenosyl, AdoCbl), methylcobalamin (R = CH3, MeCbl) and cob(II)alamin (R = e-, CblII) Center: Formula of the metal-free corrin hydrogenobyric acid (Hby), of Co(II)-cobyric acid (CbyII), zincobyric acid (Znby), with the omission of the β-axial ligands at Co(II) and Zn(II), and of the Ni(II)corrin nibyric acid (Niby). Right: formula of metal-free hydrogenobinamide (Hbi), of cob(I)inamide (CbiI) and of nibinamide (Nibi).

vitamin B_{12} analogues zincobalamin (**Znbl**) and nibalamin (**Nibl**), both also structurally characterized [15, 16].

In the Eschenmoser labs [17, 18], a nickel corrin was constructed in the 1960s as the first synthetic corrin, also allowing for an unprecedented X-ray crystallographic investigation of the structure of a non-cobalt corrin [19]. In more recent times, the quest for Ni-analogues of the B_{12}-cofactors as structural B_{12}-mimics has resurged [10, 11, 16], as Ni(II)-analogues of the cobalamins (**Cbls**) are predicted to reveal interesting coordination chemical features and could represent 'antivitamins B_{12}' [20–23]. The structural elucidation of the related natural porphyrinoid nickel-cofactor F_{430} [24] and its complex biological chemistry [25, 26] have strongly boosted interest in the basic coordination chemistry of tetrapyrrolic nickel-complexes [27–29]. Herein, we report on the metal-free cobinamide ligand hydrogenobinamide (**Hbi**) and its Ni(II)-complex nibinamide (**Nibi**) (see Scheme 1), and their first synthesis.

RESULTS AND DISCUSSION

The metal-free Cbi-ligand **Hbi** was prepared from **Hby** by attaching the (*R*)-isopropanolamine moiety to its carboxylic acid group, using an established carbodiimide method [30]. In brief, an aqueous solution of 2.92 mg (3.33 µmol) of **Hby** and of 20 moleq N-hydroxybenztriazole (HOBt), was treated with 7.96 mg (106 µmol) of (*R*)-isopropanolamine in 200 µl of 1 M HCl. The solution was degassed and frozen with external dry ice, and 2 mg (4 moleq) of EDC*HCl was added under Ar. The reaction



Scheme 2. Outline of the synthesis of **Hbi** and **Nibi** from **Hby**. i) (*R*)-1-amino-2-propanol; HOBt, EDC*HCl, in H_2O; ii) $Ni(OAc)_2$ in H_2O, 90 °C.

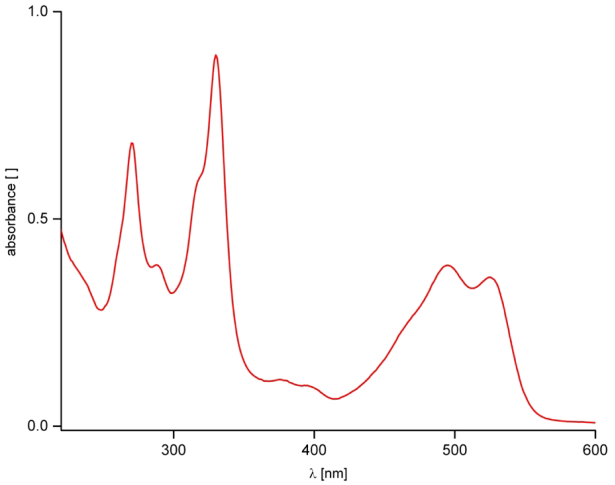


Fig. 1. UV/Vis-absorption spectrum of **Hbi** (c = 19 µM in 10 mM aq. Na-phosphate pH5, 298K).

mixture was warmed up to 0 °C and its pH was adjusted to 6.4. Further addition of six portions of about 3 moleq EDC*HCl each at 0 °C over the course of 168 h, isolation of the product using RP18 solid phase extraction and lyophilization furnished 2.88 mg (2.82 µmol, 85% yield) of pure **Hbi**-BF_4 as an orange powder (see Scheme 2).

An aqueous solution of **Hbi** at pH 5 exhibited UV/Vis- and CD-spectral features (see Fig. 1 and Fig. S2), as well as strong fluorescence with maxima at 609 nm and 553 nm (see Fig. S3), all similar to the corresponding spectra of **Hby** [7]. A high-resolution mass spectrum of **Hbi** confirmed the expected molecular formula $C_{48}H_{74}N_{11}O_8$ (see Fig. S4). The data from hetero-nuclear NMR-spectra allowed for the assignment of 73 of the 74 H-atoms and of all 48 C-atoms (see Table S2), which allowed the structure of **Hbi** in an aqueous solution to be elucidated. Two 'inner' H-atoms were detected that gave rise to singlets at δ = 12.44 and δ = 12.68 ppm in the ^{1}H-NMR spectrum (see Fig. 2). Using the critical HMBC and NOE correlations (see e.g. [31–33]), the two singlets were assigned to H(N4) and H(N2), respectively (see Fig. S5), in line with the corresponding assignments for **Hby** [7] and for the complete metal-free B_{12}-ligand hydrogeno-balamin (**Hbl)** [16], but (formally) contrasting the position of the 'inner' H-atoms in Eschenmoser's metal-free model corrin [34]. Only small shifts to the lower field by 0.01 ppm (^{1}H) and by roughly 3ppm (^{15}N) were indicated for the inner NH-groups in the spectrum of **Hbi**, when compared to **Hby** (see Tables S1 and S2). The detailed data from homo-nuclear and hetero-nuclear correlations (see Fig. S6) were consistent not only with the established diagonal arrangement of two 'inner' protons in metal-free corrins [7, 16, 34], but also with the intact stereo-structure of **Hbi**.

With pure samples of metal-free **Hbi** in hand, the one-step synthesis of transition metal analogues of the cobalt-containing cobinamides, metbinamides (**Metbi**s), has become a realistic target, providing access to a still unexplored area in the wider B_{12}-field. Thus, the Ni(II)-corrin nibinamide (**Nibi**) was prepared by heating a deoxygenated aqueous solution of **Hbi** and $Ni(OAc)_2$ pH 6 for 1 h at 90°C (Scheme 2), furnishing pure **Nibi**-BF_4 (56% yield) as a yellow powder. An aqueous solution of **Nibi** buffered to pH 5 exhibited UV/Vis- and CD-spectra very similar to the corresponding spectra of **Niby** (see Fig. 3 and Fig. S8).

The solution structure of **Nibi**, molecular formula $C_{48}H_{72}BF_4N_{11}O_8Ni$ from a high-resolution ESI-MS spectrum (see Fig. S9), was determined by hetero-nuclear

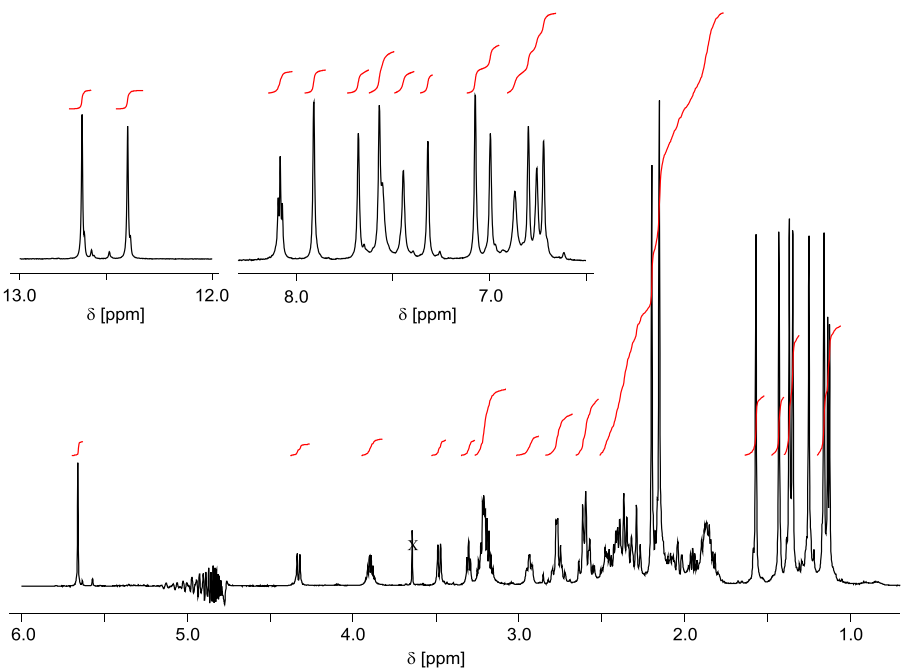


Fig. 2. 600MHz ^{1}H-NMR spectrum of **Hbi** (c = 2 mM) in H_2O/D_2O (9:1) at pH5 and 298K.

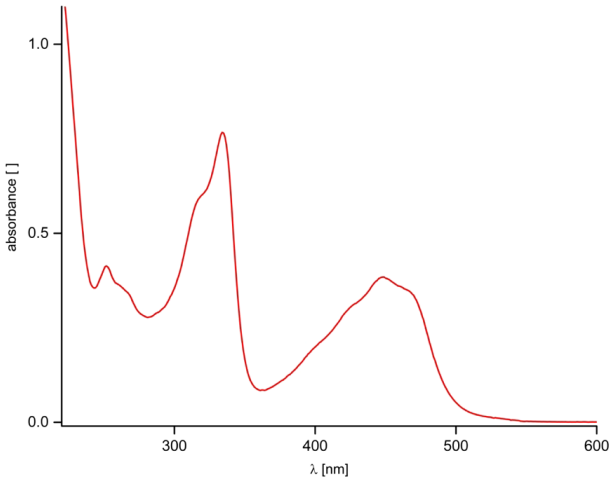


Fig. 3. UV-Vis spectrum of **Nibi** (c=44 µM) in 10 mM aqueous Na-phosphate buffer pH 5 (298K).

high field NMR-spectroscopy (see Fig. 4 for a 500 MHz ^{1}H-NMR spectrum), providing assignment of all 58 non-exchangeable H-atoms and all 48 C-atoms (see Table S3). The NMR-data for the Ni(II)-complex **Nibi** featured very similar characteristics to the ones described for **Niby**. Compared to the spectrum of metal-free **Hbi**, similar shift differences were determined from the NMR-data for **Nibi**, as had been observed earlier for the corresponding pair **Hby** and **Niby** [16]. The set of homo-nuclear and hetero-nuclear correlations from the NMR spectra of **Nibi** in D_2O also confirmed its expected stereo-structure (see Fig. S10). ^{1}H,^{1}H-NOE-correlations between the isopropanol terminus of the modified f-side chain and C151, as well as the neighboring ring D moiety of the corrin ring, support a time-averaged position of this rather lipophilic terminal group near the lower face of the corrin. In fact, the acquired spectral data suggest the diamagnetic Ni(II)-corrin **Nibi** be an *iso*-electronic and roughly

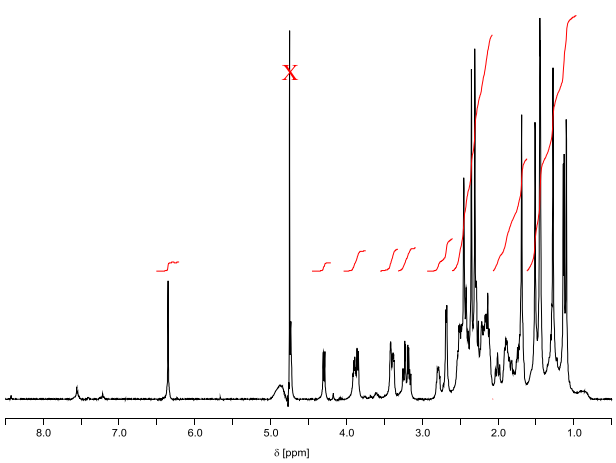


Fig. 4. 500 MHz ^{1}H-NMR-spectrum of **Nibi** (c=2.6 mM in D_2O, HDO pre-saturated, 298K) X= residual HDO signal.

iso-structural mimic of the strongly reducing [35] and highly nucleophilic, but structurally less characterized Co(I)-corrin cob(I)inamide (**Cbi^{I}**).

The major structural effects of the formal replacement of cobalt by nickel in vitamin B_{12} derivatives were revealed by the X-ray crystal structure analysis of **Niby**, finding the 4-coordinate diamagnetic Ni(II)-ion of **Niby** located close (at 0.025 Å) to the best plane through the four inner corrin N-atoms [16]. The 4-coordinate Ni(II)-ion is bound with short average Ni-N bond lengths of 1.86 Å in **Niby** [16]. This crystallographic finding, and a complementary one with the Rh(III)-corrin adenosylrhodibalamin (**AdoRhbl**) [9], support the suggestion that the coordination hole of the 'ring contracted' corrin ligand is still too large for strain-free binding of a low-spin d^8-Ni(II)-center. However, the Ni(II)-ion of **Niby** was deduced to coordinate the corrin ligand in a similar way [16] as the 4-coordinate Co(II)-center of a protein-bound Co(II)corrin [36], or as the 5-coordinate Co(II)-centers in the crystalline Co(II)-corrins **Cbl^{II}** [37] and cob(II)yrinic acid heptamethyl ester [38].

We have developed here a rational, direct synthesis path to the polar metal-free Cbi-ligand **Hbi** and to its diamagnetic Ni(II)-complex nibinamide **Nibi** (the Ni(II)-analogue of **Cbi**). Both of these novel corrins reveal key structural features of **Cbis**, the major 'incomplete' natural cobalt-corrins. This work extends our recent studies with **AdoRhbl** [9], **ClRhbl** [39] and zincobalamin (**Znbl**) [15], Rh(III)- and Zn(II)- analogues of 6- and 5-coordinate 'base-on' **Cbls**, resp., as well as with nibalamin (**Nibl**) [16], the Ni(II)-analogue of ('complete') four coordinate 'base-off' **Cbl**-forms including **Cbl^{I}**. The Ni(II)-corrin **Nibi** is presented here as an excellent redox-stable structural mimic for the corresponding natural 4-cooordinate 'incomplete' **Cbi^{II}**- and **Cbi^{I}**-species. Such activated reduced **Cbi^{II}**- and **Cbi^{I}**-species play the roles of highly reactive intermediates in basic B_{12}-biosynthetic enzyme processes in microorganisms that generate coenzyme B_{12} (**AdoCbl**) from externally supplied and actively imported **Cbi**s [40] via cobalt-adenosylation and subsequent 'completion' to Ado-cobamides [13, 41–45]. As a stable **Cbi**-mimic, **Nibi**, thus, may represent a B_{12}-antimetabolite with the potential of selectively impairing the B_{12}-biosynthetic capacity of bacteria. Like the cobinamides [41, 46], **Nibi** would be predicted to possess the little capacity to downregulate the expression of the bacterial B_{12}-uptake systems as ligands of the B_{12}-riboswitches. **Nibi** would, furthermore, not be expected to find a ready cellular import in humans and animals via their B_{12}-uptake system [47–48], contrasting with the behavior of genuine 'antivitamins B_{12}' [20–22, 49]. As a consequence, **Nibi** represents a novel antibiotic candidate, selectively targeting microorganisms. Accordingly, studies with the 'incomplete' Ni(II)-corrin **Nibi** and with other suitably structured transition metal analogues of the **Cbis** are clearly worthwhile.

EXPERIMENTAL

General

Materials

Methanol (MeOH), acetonitrile (MeCN), HiPerSolv Chromanorm, and acetic acid (HOAc) p.A., 1-hydroxybenzotriazole (HOBt), sodium hydroxide (NaOH) p.A. were from VWR chemicals; R(-)-1-amino-2-propanol from Fluka; N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC*HCl), nickel acetate trihydrate ($Ni(OAc)_2$*3 H_2O) p.A.; tetrafluoroboric acid 48% in H_2O; sodium tetrafluoroborate, p.A., sodium acetate (NaOAc); sodium dihydrogenphosphate (NaH_2PO_4), disodium hydrogenphosphate (Na_2HPO_4) from Sigma Aldrich; water (H_2O) deionized, was purified by reversed osmosis via MilliQ academic system; D_2O 99.96%D from Eurisotop; *Spectra*: UV-Vis: Agilent Cary 60. CD: Jasco J-715 or Jasco J-1500-150 CD spectropolarimeters, spectra were recorded at 298K. NMR: 500 MHz Varian Unity Inova, 5mm triple-resonance probe with z-gradients, pulse sequences from VNMR J-ChemPak 4.1; 600 MHz Bruker Avance II+ with Prodigy TCI™ probe; ^1H reference to δ(HDO) = 4.75 ppm, signal assignments were based on ^1H, (^1H,^1H)-COSY, (^1H,^{13}C)-HSQC, (^1H,^{13}C)-HMBC and (^1H,^1H)-ROESY spectra. ESI-HR-MS: Thermo Scientific LTQ-Orbitrap XL, (+)-ion mode, 4.5 kV in MeOH. *Chromatograpy*: HPLC using Hitachi Elite LaChrom, L2130 pump, L245 diode array detector; Dionex Ultimate 3000, variable wavelength detector; column: YMC-Triart –C18, 250x4.7 mm, S-5 µm, 12 nm; solvent composition: A: 10 mM aqueous NH_4OAc pH 7, B= MeOH; 8% to 95%B 0–40 min, 95% B 40–44 min, 95% to 8%B 44–45 min, flow= 1 mL/min. RP18-MPLC: Büchi C-605 pump module (binary) flow≈10 mL/min, home-packed RP18 column (l=230 mm, Ø=26 mm, column volume (*cv*) = 122 mL) using about 100 g LiChroprep RP18. Sep-Pak® C18 cartridges (various sizes, from Waters) were conditioned with 20 mL MeOH and 60 mL H_2O prior to use.

Synthesis of hydrogenobinamide tetrafluoroborate (Hbi)

In a 20 mL 2-necked round bottom flask 2.92 mg (3.33 µmol) **Hby** [7], 8.88 mg (65.7 µmol, 20 eq) 1-hydroxybenzotriazole (HOBt) were dissolved in 3.2 mL H_2O. A solution of 7.96 mg (106µmol, 32eq) R-(-)-1-amino-2-propanol in 100 µL 1 M HCl was added and the mixture was deoxygenated by 3 freeze/vacuum/thaw cycles. The solution was frozen and 2.0 mg (10.6 µmol, 4eq) N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC*HCl) were added under Ar. The solution was thawed and the pH was adjusted to pH 6.4 with ~40µl 1 M NaOH under vigorous stirring. The reaction was kept on ice for 7d and ~1.5–2 mg (~3eq) EDC*HCl were added once per day in Ar counter flux. After 168 h the orange reaction solution was diluted with H_2O to ~10ml and loaded on a Sep-Pak® plus long cartridge. The adsorbate was washed with 20 mL H_2O, 20 mL 100 mM aqueous $NaBF_4$ pH 6, and a further 20 mL H_2O. The **Hbi** was eluted with 3 mL 100 µM $NaBF_4$ in MeOH. The eluate was frozen and lyophilized under HV. The residue was dissolved in 1ml H_2O and lyophilized again. 2.88 mg (2.82 µmol, 85%) of powdery orange **Hbi** (a tetrafluoroborate) were obtained (for HPLC see Fig. S1). UV/Vis (c=18.9 µM in 10 mM aq. Na-phosphate pH 5, RT): λ^{max} [nm] (lg ε) = 525 (4.28), 495 (4.31), 472 (sh, 4.16), 3.93 (3.71), 377 (3.77), 330 (4.67), 320 (sh, 4.51), 288 (4.31), 270 (4.56) (see Fig. 1). CD (c=42.4 µM in 10 mM aq. Na-phosphate pH 5, 293K): $\lambda^{max/min}$ [nm] (± Δε [l*mol^{-1}*cm^{-1}]) = 522 (-3.5), 501 (-2.7), 394 (0.4), 328 (15.2), 270 (-7.9), 232 (3.1); λ^0 [nm] = 425, 367, 296, 242, 222 (see Fig. S2). Fluorescence (c=7.62 µM in 10 mM aq. Na-phosphate pH 5): emission spectrum (λ^{exc} = 500 nm): λ^{max} [nm] (rel. int.) = 609 (406), 553 (216); excitation spectrum (λ^{em} = 609 nm): λ^{max} [nm] (rel. int.) = 526 (477), 502 (413), 393 (70), 376 (81), 330 (580), 320 (439), 305 (285), 270 (394), 262 (258) (see Fig. S3). ^1H-NMR spectra were measured at 600 MHz (c(Hbi) = 1.96 mM in 10 mM Na-phosphate in 10% D_2O, at 298K, see Fig. 2), ^1H- ^{13}C- and ^{15}N-signal assignments from 2D homo- and hetero-nuclear spectra, see Figs. S5 and S6, Tables S1 and S2). HR-ESI-MS (MeOH): 934.579 (12), 933.575 (57), 932.572 (100, $[C_{48}H_{74}N_{11}O_8]^+ \equiv [M]^+$); 486.269 (5), 485.767 (10, $[M+K]^{2+}$); 478.783 (16), 478.281 (56), 477.780 (98, $[M+Na]^{2+}$); 467.291 (9), 466.789 (16, $[M+H]^{2+}$) (see Fig. S4).

Synthesis of nibinamide tetrafluoroborate (Nibi)

In a 15 mL Schlenk tube equipped with a reflux condenser 2 mL 0.5 M $Ni(OAc)_2$ pH were degassed by 5 freeze/vacuum/thaw cycles. 1.70 mg (1.82 µmol) **Hbi** were added and the mixture was degassed by further 3 freeze/vacuum/thaw cycles. The apparatus was pressurized with Ar and the brown solution was heated to 90°C for 1h. After cooling to room temperature the apparatus was aerated and the green solution was diluted with H_2O to 20 mL. The solution was loaded on a Sep-Pak® C18 Classic cartridge. The adsorbate was washed with 20 mL H_2O followed 20 mL 100 mM $NaBF_4$ pH 6 and a further 20 mL H_2O. The crude nibinamide (**Nibi**) was eluted with 3 mL 100 µM $NaBF_4$ in MeOH. The solvents were evaporated on the rotary evaporator (55°C), and the residue was dissolved in 10 mM NaOAc pH 6 and loaded on the MPLC column. The crude **Nibi** was purified using 1 L portions of 10%, 12%, 13%, 14%, 15%, and 16% MeCN in 10 mM $Na(OAc)_2$. The Nibi-containing fraction was concentrated to ~40 ml on the rotary evaporator (50°C) and loaded on a Sep-Pak® C18 Classic cartridge. The adsorbate was washed with 80 mL 100 mM $NaBF_4$ pH 6, 20 mL 50 mM $NaBF_4$, and 20 mL H_2O. The fraction

with **Nibi** was eluted with 2 mL 100µM $NaBF_4$ in MeOH and evaporated on the rotary evaporator. The residue was dissolved in 1 mL of H_2O and the sample was lyophilized overnight. 1.10mg (1.02µmol, 56%) of yellow powdery **Nibi** were isolated pure (for HPLC see Fig. S7). UV-Vis (c=44.1µM in 10mM Na-phosphate pH 5, RT): λ^{max} [nm] (lg ε) = 465 (3.90), 448 (3.94), 430 (sh, 3.86), 404 (sh, 3.69), 334 (4.24), 321 (sh, 4.14), 262 (sh, 3.91), 252 (3.97) (see Fig. 3). CD (c=44.1µM in 10mM Na-phosphate pH 5, 293K): $\lambda^{max/min}$ [nm] ($\pm\Delta\varepsilon$ [l*mol^{-1}*cm^{-1}]) = 457 (-0.79), 413 (0.53), 326 (sh, 3.35), 315 (4.94), 255 (-5.86); λ^0 [nm]= 431, 393, 342, 289, 226 (see Fig. S8). ^{1}H-NMR spectra were measured at 500 MHz (c(Nibi) =2.55 mM in D_2O at 298K, see Fig. 4), ^{1}H- and ^{13}C-signal assignments from 2D homo- and hetero-nuclear spectra, see Fig. S10 and Table S3). HR-ESI-MS (MeOH): m/z (%)=992.944 (2), 991.490 (20), 990.488 (34), 989.495 (55), 988.492 (100, $[C_{48}H_{72}N_{11}NiO_8]^+ \equiv [M]^+$); 513.726 (5, $[M+K]^{2+}$); 507.740 (3), 507.239 (17), 506.738 (32), 506.21 (42), 505.739 (76, $[M+Na]^{2+}$); 496.248 (7), 495.747 (15), 495.250 (20), 494.749 (37, $[M+H]^{2+}$) (see Fig. S9).

Acknowledgments

This work was supported by grants from the Austria Science Fund (FWF, P-28892 and P33059) to BK and from the Biotechnology and Biological Sciences Research Council (BBSRC) BB/S002197/1 to MJW.

Supporting information

Figures of CD-, fluorescence and mass spectra, and NMR-data are given in the supplementary material. This material is available free of charge *via* the Internet at https://www.worldscientific.com/doi/suppl/10.1142/S1088424623500463

REFERENCES

1. Eschenmoser A. *Angew. Chem. Int. Ed.* 1988; **27**: 5–39.
2. Pratt JM. *Inorganic Chemistry of Vitamin B_{12}*, Academic Press, New York, 1972.
3. da Silva JJRF and Williams RJP. *The Biological Chemistry of the Elements*, Clarendon Press, Oxford, 1991.
4. Gruber K, Puffer B and Kräutler B. *Chem. Soc. Rev*. 2011; **40:** 4346–4363.
5. Osman D, Cooke A, Young TR, Deery E, Robinson NJ and Warren MJ. *Biochim. Biophys. Acta* 2021; **1868**: 118896.
6. Blaser HU, Winnacker EL, Fischli A, Hardegger B, Bormann D, Hashimoto N, Schossig J, Keese R and Eschenmoser A. *Helv. Chim. Acta* 2015; **98**: 1845–1920.
7. Kieninger C, Deery E, Lawrence AD, Podewitz M, Wurst K, Nemoto-Smith E, Widner FJ, Baker JA, Jockusch S, Kreutz CR, Liedl KR, Gruber K, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2019; **58**: 10756–10760.
8. Koppenhagen VB. *Metal-Free Corrinoids and Metal Insertion*, in: D. Dolphin (Ed.) B_{12}, John Wiley & Sons, 1982, pp. 105–150.
9. Widner FJ, Lawrence AD, Deery E, Heldt D, Frank S, Gruber K, Wurst K, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed*. 2016; **55**: 11281–11286.
10. Holze G and Inhoffen HH. *Angew. Chem. Int. Ed.* 1985; **24**: 867–869.
11. Brenig C, Prieto L, Oetterli R and Zelder F. *Angew. Chem. Int. Ed.* 2018; **57**: 16308–16312.
12. Battersby AR. *Nat. Prod. Rep.* 2000; **17**: 507–526.
13. Bryant DA, Hunter CN and Warren MJ. *J. Biol. Chem*. 2020; **295**: 6888–6925.
14. Deery E, Schroeder S, Lawrence AD, Taylor SL, Seyedarabi A, Waterman J, Wilson KS, Brown D, Geeves MA, Howard MJ, Pickersgill RW and Warren MJ. *Nat. Chem. Biol*. 2012; **8**: 933–940.
15. Kieninger C, Baker JA, Podewitz M, Wurst K, Jockusch S, Lawrence AD, Deery E, Gruber K, Liedl KR, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2019; **58**: 14568–14572.
16. Kieninger C, Wurst K, Podewitz M, Stanley M, Deery E, Lawrence AD, Liedl KR, Warren MJ and Kräutler B. *Angew. Chem. Int. Ed.* 2020; **59**: 20129–20136.
17. Bertele E, Boos H, Dunitz JD, Elsinger F, Eschenmoser A, Felner I, Gribi HP, Gschwend H, Meyer EF, Pesaro M and Scheffold R. *Angew. Chem. Int. Ed. Engl.* 1964; **3**: 390–496.
18. Bertele E, Scheffold R, Gschwend H, Pesaro M, Fischli A, Roth M, Schossig J and Eschenmoser A. *Helv. Chim. Acta* 2015; **98**: 1755–1844.
19. Dunitz JD and Meyer jun. EF, *Helv. Chim. Acta* 1971; **54**: 77–89.
20. Kräutler B. *Chem. Eur. J.* 2015; **21**: 11280–11287.
21. Kräutler B. *Chem. Eur. J.* 2020; **26**: 15438–15445.
22. Kräutler B. *Antivitamins B_{12}*, in: Litwack G (Ed.) *Vitamins and Hormones*, Academic Press, Cambridge, Mass., 2022, pp. 221–240.
23. Zelder F, Sonnay M and Prieto L. *ChemBioChem* 2015; **16**: 1264–1278.
24. Pfaltz A, Jaun B, Fässler A, Eschenmoser A, Jaenchen R, Gilles HH, Diekert G and Thauer RK. *Helv. Chim. Act*a 1982; **65**: 828–865.
25. Jaun B and Thauer RK. *Nickel-Alkyl Bond Formation in the Active Site of Methyl-Coenzyme M Reductase*, in: Sigel A, Sigel H and Sigel RKO (Eds.) Metal-Carbon Bonds in Enzymes and Cofactors, Royal Society of Chemistry, Cambridge, 2009, pp. 115–132.
26. Scheller S, Goenrich M, Boecher R, Thauer RK and Jaun B. *Nature* 2010; **465**: 606–609.

27. Kratky C, Waditschatka R, Angst C, Johansen JE, Plaquevent JC, Schreiber J and Eschenmoser. *Helv Chim Acta* 1985; **68**: 1312–1337.
28. Kraatz HB and Metzler-Nolte N. *Concepts and Models in Bioinorganic Chemistry*, Wiley VCH, Weinheim, 2006.
29. Wicht R, Bahnmuller S, Brandhorst K, Schweyen P and Bröring M. *Chem. Sci.* 2016; **7**: 583–588.
30. Widner FJ, Gstrein F and Kräutler B. *Helv. Chim. Acta* 2017; **100**: e1700170
31. Tollinger M., Konrat R and Kräutler B. *Helv. Chim. Acta* 1999; **82**: 1596–1609.
32. Summers MF, Marzilli LG and Bax A. *J. Am. Chem. Soc.* 1986; **108**: 4285–4294.
33. Bax A, Marzilli LG and Summers MF. *J. Amer. Chem. Soc*. 1987; **109**: 566–574.
34. Edmond ED and Hodgkin DC. *Helv. Chim. Acta* 1975; **58**: 641–654.
35. Lexa D and Savéant JM. *Acc. Chem. Res*. 1983; **16**: 235–243.
36. Kräutler B, Keller W and Kratky C. *J. Am. Chem. Soc*. 1989; **111**: 8936–8938.
37. St Maurice MS, Mera P, Park K, Brunold TC, Escalante-Semerena JC and Rayment I. *Biochemistry* 2008; **47**: 5755–5766.
38. Kräutler B, Keller W, Hughes M, Caderas C and Kratky C. *J. Chem. Soc., Chem. Comm*. 1987; 1678–1680.
39. Widner FJ, Kieninger C, Wurst K, Deery E, Warren MJ and Kräutler B. *Synthesis* 2021; **53**: 332–337.
40. Bradbeer C. *Cobalamin Transport in Bacteria*, in: Banerjee R (Ed.) Chemistry and Biochemistry of B_{12}, John Wiley & Sons, New York, 1999, pp. 489–506.
41. Kennedy KJ, Widner FJ, Sokolovskaya OM, Innocent LV, Procknow RR, Mok KC and Taga ME. *mBio* 2022; https://doi.org/10.1128/mbio.01121–22.
42. Escalante-Semerena JC, Woodson JD, Buan NR and Zayasm CL. *Conversion of Cobinamide into Coenzyme B_{12}*, in Warren MJ and Smith AG (Eds.) Tetrapyrroles: Birth, Life and Death, Landes Bioscience, Austin, Texas, 2008, pp. 298–314.
43. Mok KC, Sokolovskaya OM, Nicolas AM, Hallberg ZF, Deutschbauer A, Carlson HK and Taga ME. *mBio*, 2020; **11**: e02507–20.
44. Gude S, Pherribo GJ and Taga ME. *Msystems* 2022; **7**: 00288–00222.
45. Jeter VL, Mattes TA, Beattie NR and Escalante-Semerena JC. *Biochemistry* 2019; **58**: 951–964.
46. Gallo S, Oberhuber M, Sigel RKO and Kräutler B. *ChemBioChem* 2008; **9**: 1408–1414.
47. Fedosov SN, Fedosova NU, Kräutler B, Nexo E and Petersen TE. *Biochemistry* 2007; **46**: 6446–6458.
48. Ruetz M, Gherasim C, Fedosov SN, Gruber K, Banerjee R and Kräutler B. *Angew. Chem. Int. Ed*. 2013; **52**: 2606–2610.
49. Mutti E, Ruetz M, Birn H, Kräutler B and Nexo E. *Plos One* 2013; **8**: e75312.